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Toxicity of Fenitrothion to Fathead Minnows (Pimephales promelas) and Alternative Exposure Duration Studies with Fenitrothion and Endosulfan

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The objectives of this study were to determine the toxic effects of fenitrothion to fathead minnows (Pimephales promelas), to investigate effects of short-term exposures to fenitrothion and endosulfan on this species, and to determine if these compounds caused delayed mortality. Fenitrothion significantly reduced survival of fathead minnows at 0.86 and 0.74 mg/l during two 14-day embryo-larvae studies. In the 30-day embroy-larvae study, growth (weight) was not adversely affected at 0.13 mg/l; however significant differences occurred at 0.30 mg/l. Growth differences were more significant than survival. A 96-hr acute value for endosulfan was 1.32 µg/l (1.13-1.54) which agrees with published values. In this study, no delayed mortality occurred.

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Toxicity of Fenitrothion to Fathead Minnows (*Pimephales promelas*) and Alternative Exposure Duration Studies with Fenitrothion and Endosulfan

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Abstract. The objectives of this study were to determine the toxic effects of fenitrothion to fathead minnows (*Pimephales promelas*), to investigate effects of short-term exposures of fenitrothion and endosulfan on this species, and to determine if these compounds caused delayed mortality. Fenitrothion significantly reduced survival of fathead minnows at 0.86 and 0.74 mg/L during two 14-day embryo-larvae studies. In the 30-day embryo-larvae study, growth (weight) was not adversely affected at 0.13 mg/L; however significant differences occurred at 0.30 mg/L. Growth differences were more significant than survival. A 96-hr acute value for endosulfan was 1.32 μg/L (1.13-1.54) which agrees with published values. In this study, no delayed mortality occurred.

Gauging the toxic levels and effects of pesticides is complicated by intermittent applications and varied dosage regimes. Further, the process of pesticide transfer from application site to the aquatic system is not fully understood.

Direct evidence of surface water contamination through aerial drift, volatilization, and percolation is as yet lacking (Haith and Tubbs 1981). However, contamination through cropland runoff has been documented and a method of measurement developed (Wauchope and Leonard 1980). Measurements show average pesticide levels in runoff to be relatively small (Haith and Tubbs 1981); however, acute sensitivity of various aquatic species varies dramatically. The objectives of this study were: 1) to produce laboratory data that would contribute to the existing data base for fenitrothion, and 2) to develop test methods for laboratory use that would closely parallel actual field exposure of non-target organisms to pesticides.

Fenitrothion was selected because it has a relatively extensive data base. Data indicate that its presence in runoff is of a high-level, short-term nature (Flannagan 1973; Morrison and Wells 1981; Lockhart *et al.* 1973). Consequently, the traditional 30-day exposure test would not accurately predict its effect on the environment. Laboratory test methods are needed that show the effects of high-level concentrations of short duration (i.e., ≤24 hr).

Solubility characteristics of fenitrothion limited the concentration that could be achieved in a flow-through system. Endosulfan overcame this difficulty and was therefore selected for further method testing due to its ease of handling and ready availability at the laboratory.

The 30-day embryo-larvae study was compared to the effects reported by Flannagan (1973), Lockhart *et al.* (1973), and Morrison and Wells (1981) to determine the effects of fenitrothion at and below levels reported in their studies and to determine whether or not delayed mortality occurs in aquatic vertebrate species.

The short-term exposures parallel work done by Abel (1980), whose studies on *Gammarus pulex* L. show delayed mortality with lethal levels of lindane and copper. Delayed mortality with fenitrothion on stream invertebrates was reported by Flannagan (1973) and in studies on coho salmon with dioxin by Miller *et al.* (1973). This study also investigates the possibility of delayed mortality occurring in fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*) when exposed to short-term, high-level doses of fenitrothion and endosulfan.

Materials and Methods

Fenitrothion

The basic system for all flow-through tests was similar to the one described below for the 30-day embryo-larvae test. Deviations from this system are later delineated for individual studies.

Physical and Chemical Conditions

A continuous-flow mini-diluter system installed in a vented enclosure (Benoit *et al.* 1982b) delivered five concentrations of toxicant plus control water into four replicate glass exposure chambers (18.7 × 7 × 9.2 cm, capacity 500 ml, water depth 5 cm). Delivery tubes positioned by stratified random assignment delivered 15 ml test water per min. Replacement took place at the rate of 90% in 85 min (Sprague 1969). Cool White and Vita Lux fluorescent lamps simulated 16-hr of daylight at an intensity of 280 lumens (242–366).

Twenty-four glass exposure jars (Mount 1968) were attached to a rotating tilt bar apparatus described by Benoit *et al.* (1982a). These jars with screened bottoms allowed for water circulation around the larvae while containing them for easy removal.

Toxicity tests were carried out in temperature controlled (23.8 ± 1°C) Lake Superior water. Total hardness, alkalinity, and pH were determined on randomly selected chambers once weekly. Dissolved oxygen was similarly measured biweekly. Test water had the following means: hardness, 47 mg/L; alkalinity, 43 mg/L; dissolved oxygen concentration, 7.58 mg/L. The pH ranged from 7.3 to 7.8.

Fenitrothion (*O,O*-dimethyl-*O*-(4-nitro-*m*-tolyl) phosphorothioate; CAS 122-14-5) solution was prepared from technical grade Sumithion® obtained from the Stauffer Chemical Co., Richmond, CA (lot #2616), with a purity of 97.4%. Water samples containing fenitrothion were extracted every 48-hr with hexane and analyzed by gas chromatography. Recovery from spiked control water samples averaged 91% (n = 13).

Saturated solutions of fenitrothion in water were used as the toxicant source to avoid the use of solvents. Solutions were prepared with a chemical saturator similar to the one described by Gingerich *et al.* (1979). Stock solutions were delivered from the saturator to the diluter by means of an FMI[1] metering pump and stainless steel tubing.

A combination of 96-hr LC50 values reported by Johnson and Finley (1980) and values derived from preliminary tests were used to establish concentrations for the embryo-larvae study (ELS). The lowest concentration which caused abnormal behavior such as erratic swimming, lethargy, or loss of orientation in the preliminary tests was used as the highest concentration in the ELS.

Test Animals: Fathead minnow eggs were obtained from the culture unit of the Environmental Research Laboratory-Duluth. From 1 to 12-hr after spawning, embryos were removed to 25°C diluent water for hardening and temperature acclimation. During this period, embryos were viewed through a dissecting scope. Debris, abnormal embryos, and embryos that were together in clumps of four or more were discarded.

[1] Fluid Metering, Inc., Oyster Bay, NY 11771

Embryo Larvae Study (ELS)

Two embryo-larvae tests were run with fenitrothion. In the first test, 30 apparently healthy embryos (6 to 12 hr old) were placed in each of 24 glass exposure jars attached to the rotating tilt bar. Exposure jars were immersed in fenitrothion solutions at concentrations of 1 mg/L or less or into control water. Dead embryos were removed daily during the hatch period. At completion of hatch, larvae were placed into the larger chambers in groups of 15 for continuous exposure until test termination.

Fish were fed equal amounts of live San Francisco Bay brine shrimp nauplii three times daily on weekdays and two times daily on weekends. Dead fish were removed daily. When survival in any chamber was reduced by 25, 50, or 75%, food was reduced correspondingly. Survival during the first two weeks of the testing period was determined by daily counts of the fish remaining in each chamber. This test was terminated after 14 days.

A second test was made to study the survival of fish at the five previous concentrations for 31 days. A survival count was made after 14 days and at 31 days so that the data could be compared. After recording the number of survivors, the fish were killed in ice water, and each individual fish was weighed. The pooled mean weights of individual fish from the second ELS were subjected to one-way analysis of variance and Dunnett's test ($P = 0.05$). Survival data expressed as percentages were transformed to arcsin $\sqrt{\%}$ (Steele and Torrie 1960) and also subjected to one-way analysis of variance and Dunnett's test ($P = 0.05$).

Short-Term Static Exposures

The objective was to determine the expected time to mortality of 50% of the organisms (ExpT 50) exposed to various concentrations of fenitrothion. Exposure times were selected by determining durations resulting in 0 and 100% mortality, then testing time periods between at progressively smaller intervals to establish a mortality-response curve. Groups of 10 fish were exposed to selected concentrations for different lengths of time, then they were transferred to clean water and observed for recovery over a period of 120-hr.

To overcome flow-through saturation limitations imposed by fenitrothion, an alternative approach using static concentrations at higher levels was conducted on both larvae and juveniles. In this test two-liter glass containers were used as exposure chambers for both larvae and juveniles. Toxicant solution was siphoned from the saturator and mixed with Lake Superior water to desired concentrations.

Ten larvae were placed by eyedropper into 120 ml glass jars with screened bottoms which were suspended in the toxicant solution. After exposure, larvae were removed carefully, avoiding impingement of larvae on the screens. Juveniles were netted and placed in groups of ten into the exposure containers. After exposure, juveniles were transferred by net to clean water for monitoring.

All flow-through recovery chambers containing larvae and juveniles were filled with control water. Fish were fed live brine shrimp. Fish were counted at 24, 48, 72, and 96-hr, and were observed up to 14 days post-exposure to determine whether delayed mortality occurred.

Chemical determinations were made throughout the study. Controls were run simultaneously to assure that static conditions did not affect survival and, in cases where this occurred, data were discarded.

Endosulfan

Three flow-through tests (a 96-hr continuous exposure and three short-term exposures) were made to study possible alternative laboratory exposure methods and to compare the results with some results reported for traditional test methods using endosulfan. Additions and adjustments to the original exposure system are listed below.

Physical and Chemical Conditions

An additional three-tiered clean water containment structure was built adjacent to the mini-diluter which housed 24 tanks on each tier.

An endosulfan solution (6,7,8,9,10,10 - hexachloro-1,5,5a,6,9,9a-hexa-hydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide; CAS 115-29-7) was prepared with Thiodan®, a solution consisting of endosulfan I and II isomers. The compound, 23.5% by weight, was obtained from the Chevron Chemical Co. (Ortho Co.).

Immobilizing levels of endosulfan at several concentrations were set up within the mini-diluter system. The solution was pumped as previously described from a stock bottle, which was renewed each week.

Test Animals: Test organisms from the culture units in the laboratory were 30 to 40-day old fathead minnows, less than 24-hr old fathead minnow larvae, and 80 to 90-day old bluegills (*Lepomis macrochirus*).

96-Hr Continuous Exposure

A 96-hr continuous exposure was conducted with a control plus five concentrations using 30 to 40-day old fathead minnows. Observations of the animals in each chamber were made at 24, 48, 72, and 96 hr. Deaths were recorded and an LC50 was calculated, using the Trimmed Spearman-Karber Method (Hamilton *et al.* 1977).

Short-Term Flow-Through Exposure

Thirty-day old fathead minnows and 80-day old bluegills were assigned to four groups of 10 organisms each and placed in exposure chambers. After exposure, the entire chamber was moved to the clean water containment structure where replacement of toxicant solution took place at a rate of 90% replacement in 85 min (Sprague 1969). Bluegill chambers were covered to prevent fish from jumping out.

All aspects of the fathead minnow larvae study were the same as those just described except that groups of 10 larvae were placed in screened bottom jars on the rotating tilt-bar within the mini-diluter. After exposure, the entire jar was transferred to toxicant-free water. Recovery periods were similar to those in fenitrothion static exposures.

These tests were repeated in order to obtain sufficient data that could be subjected to the Trimmed Spearman-Karber Method of analysis. The ExpT 50 was computed at each concentration for each sample set.

Table 1. Expected time to death (in hours) for 50% of organisms (ExpT 50) exposed to fenitrothion for various time periods

Test species (Age)	Fenitrothion concentration (mg/L)	ExpT 50	
		24 hr	120 hr
Fathead minnow			
<24 hr old fry	20[b]	5.88	5.04
	15	6.06	6.06
	13.5[b]	9.84	
	9.25[a]	5.39	5.08
	9[b]	12.7	11.31
	8.2[b]	21.08	
	3.3	32.60	32.60
30+ day old juvenile	11[b]	1.00	50
	9[b]	4.58	25

[a] Measured average concentration (unlabeled are actual concentrations)
[b] Nominal concentrations

Results

Fenitrothion

Embryo-Larvae Study: Survival of test fish was significantly reduced at 0.86 and 0.74 mg/L, respectively. A comparison of survival at the various exposure concentrations on the 14th day in both studies and on the 31st day of the continuation study indicated little difference.

Growth in weight was not adversely affected at 0.13 mg/L; however, significant reduction occurred at 0.30 mg/L and at 0.74 mg/L.

Short Exposure Studies: Expected times to kill 50% from exposures to fenitrothion are reported in Table 1. Larvae frequently died after six days, which is the expected time of larvae starvation (Benoit *et al.* 1982a). Concentrations of fenitrothion are ranked in descending order. Most ExpT 50 values are for 24-hr old larvae at start, and due to expected mortalities, appear to be valid for only six days or 120 hr. At a concentration of 3.3 mg/L, reported ExpT 50 occurs after the 24-hr observation period. However, due to the Trimmed Spearman-Karber Method requirements, values taken at 48 hr were used and reported.

Many fish that showed signs of stress during the exposure period recovered completely after being removed to clean water. Most fish that died did so in the first 24 hr. This test showed no evidence of delayed mortality.

Endosulfan

96-Hr Continuous Exposure: Effects were seen at

Table 2. Expected time to death (in hours) for 50% of organisms (ExpT 50) exposed to endosulfan for various time periods

Test species (Age)	Endosulfan concentration (μg/L)	ExpT 50	
		24 hr	14 day
Fathead minnow			
<24 hr fry	35	1.74	
30+ day old	31.8	1.78	1.78
juveniles	17.2	1.67	1.49
	15.2[a]	4.04	4.04
	12.9[a]	3.74	3.74
	10.5[a]	3.64	3.64
	6.1[a]	5.78	5.78
	5.4[a]	8.90	8.90
	3.0	19.5	16.13
	1.1[a]	37.97	37.97
Bluegills			
(80–90 day old)	21.7	1.84	1.84
	17.2	1.13	1.13
	9.4[a]	4.84	4.64
	8.7[a]	4.24	4.24
	6.5[a]	9.24	9.24
	2.2[a]	20.78	20.51

[a] Measured average concentration (all others are actual concentrations)

several concentrations. For comparison, fathead juvenile mortalities in the 1.2 and 2.4 μg/L concentrations allowed calculation of 24, 48, 72, and 96-hr LC50 values. These values with 95% confidence intervals, are 1.84 (1.65–2.05) for 24 hr, 1.70 for 48 hr, 1.32 (1.13–1.54) for 72 hr, and 1.32 (1.13–1.54) μg/L for 96 hr of exposure.

Short-Term Exposure: ExpT 50 values for juveniles and bluegills are reported in Table 2 and are plotted against concentrations for all reported data. Recovery of survivors was rapid when fish were transferred to pesticide-free water. Within 24 hr, all were able to swim normally. Nearly all fish that died did so during the exposure period. Only three mortalities in over 600 organisms occurred during the post-exposure period. This resulted in consistently identical ExpT 50 values computed after 14 days of post-exposure holding.

Discussion

Fenitrothion

Growth was the best indicator of fenitrothion effects in the 31-day embryo-larval study. The estimated maximum acceptable toxicant concentration in this study was between 0.13 and 0.30 mg/L based on reduced weight. Survival in the first 14-day exposure was not affected at 0.40 mg/L, but was significantly reduced ($P < 0.05$) at 0.86 mg/L. Survival in the second 14-day and in the 31-day exposures was not affected at 0.30 mg/L, but was significantly reduced ($P < 0.05$) at 0.74 mg/L (Table 3). These results agree with Benoit *et al.* (1982a), who showed that survival rates after two weeks of exposure to four organic chemicals accurately predict survival at four weeks.

Johnson and Finley (1980) reported 96-hr LC50 values for fenitrothion and fathead minnows of 3.2 with a 95% confidence interval of 2.4–4.2 mg/L and 4.8 with a confidence interval of 3.8–6.1 mg/L. They also reported 96-hr LC50 values for several salmonids of 2.0–2.4 mg/L. These values are similar to those reported for fathead minnows.

Seldom if ever do field concentrations reach these levels. Studies done by Flannagan (1973), Lockhart *et al.* (1973), and Morrison and Wells (1981) show that fenitrothion flushes in approximately 24 hr, dropping to negligible levels at the end of five days. Data in Table 1 show that water concentration values of 20 mg/L fenitrothion would have to exist for five-plus hr before one would see 50% of the fathead minnow population destroyed. Lockhart reported the highest field value of 75.8 μg/L in a static pool within his field study area.

High variability in the values reported in Table 1 was partially due to the use of a static solution to maintain high concentration levels. Toxicity levels exceeded the saturation value of 1 mg/L as reported by Wauchope (1978). It was difficult to establish a range of concentrations in sufficient volume to perform short exposure testing at or close to saturation. In addition, while most of the testing was done with larvae, juveniles were used in later testing in an attempt to overcome limitations posed by larvae. These limitations included deaths due to starvation (Benoit *et al.* 1982a), stress and hemorrhage during handling, and small size of larvae which limited the opportunity to observe subtle effects of the toxicant.

In tests performed with juveniles, behavior changes could be noted in greater detail. These changes included an initial increase in activity followed by disorientation, loss of equilibrium, and finally, apart from slight opercular movements, an inactive state. This effect was seen in more than 50% of the survivors of the two data sets reported, as well as in several other data sets that did not lend themselves to analysis by the Spearman-Karber method. Lockhart *et al.* (1973) reported effects with rainbow trout at 4.6 mg/L. Though all of his fish were similarly affected, none of them died during the seven-month post observation period.

When the juveniles were most seriously affected

Table 3. Survival and growth of fathead minnows chronically exposed to fenitrothion

Test 1					
Mean measured water concentration (mg/L, n = 6)					
0.000	.04 ± .01[a]	.09 ± .01	.19 ± .02	.40 ± .04	.86 ± .09
% Survival (14 day)[a]					
59 ± 28[a]	74 ± 24	76 ± 11	62 ± 10	68 ± 7	29 ± 3[c]
Test 2					
Mean measured water concentration (mg/L, n = 12–14)					
0.000	.02 ± .01	.06 ± .02	.13 ± .04	.30 ± .09	.74 ± .19
% Survival (14 day)[b]					
72 ± 13[a]	83 ± 9	76 ± 11	80 ± 13	63 ± 5	31 ± 5[c]
% Survival (31 day)[b]					
74 ± 13	83 ± 9	72 ± 12	77 ± 15	60 ± 8	29 ± 6[c]
Pooled growth (weight, mg)					
145 ± 43	141 ± 38	148 ± 41	138 ± 35	102 ± 40[c]	58 ± 44[c]

[a] Mean and standard deviation
[b] Numbers surviving subjected to arcsin $\sqrt{\%\ \text{survival}}$ eliminating fish lost to mechanical error (15 fish/replicate)
[c] Significantly different from control ($P \leq 0.05$)

by the toxicant, they were removed to clean water. Within 24 hr, behavior had apparently returned to normal. Recovery appeared to be complete. Morrison and Wells (1981) reported uptake values for several aquatic vertebrate species which appear to parallel the rise and fall of water toxicant levels with fenitrothion.

Endosulfan

The 96-hr LC50 of 1.32 μg/L in this study is similar to those reported by Lemke (1981) who reported 96-hr LC50 values of 0.99 (0.29–1.91) μg/L for fathead minnows in a round-robin of several laboratories. Johnson and Finley (1980) reported LC50 values of 1.5 (1.1–2.0) μg/L for fathead minnows and 1.2 (0.9–1.7) μg/L for bluegills. Macek (1976) reported 30 day embryo-larval values of >0.20 and <0.40 μg/L.

Field studies similar to those done by Flannagan (1973) and Morrison and Wells (1981) were conducted by Fox and Matthiessen (1982) who sprayed for tsetse fly control in Africa at 0.96 g/ha. Endosulfan concentrations ranged from 0.2–4.2 μg/L six to nine hr after application.

Assuming that peak concentrations were limited to nine hr, comparisons with the former laboratory derived values would indicate that fish exposed under these peak concentration conditions would be minimally affected. At concentrations of 2-3 μg/L for 20 hr, mortalities would be expected to occur with both fathead minnows and bluegills (Table 2). ExpT 50 values for both fathead minnows and bluegill were nearly the same at 14 days as they were at one day.

Delayed mortality did not occur in either the fenitrothion or endosulfan studies. Fish that remained alive at 24 hr survived the six day observation period in the fenitrothion study, and those that were alive at 24 hr survived the 14-day observation period in the endosulfan study. The mortalities that did occur in larvae were probably due to starvation rather than to toxicity. This agrees with Morrison and Wells' (1981) and Lockhart *et al.* (1973) exposure of trout to fenitrothion in which they held fish for three and seven months, respectively, post-exposure, and saw no mortalities.

These test methods hold promise, but a wider data base is needed to better evaluate their utility. Their usefulness will be limited until the transfer process of pesticides from application site to the aquatic system is better understood.

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